Tumor blood flux quantification using flow enhanced MRI and comparison with histology

Olivier Reynaud[1], Francoise Geffroy[1], and Luisa Ciobanu[1]
[1]*NeuroSpin, Saclay, France*

Target audience: Clinical and preclinical researchers interested in new tools for perfusion and/or tumor characterization

Introduction: Non-invasive quantitative assessment of brain microvasculature is important for the diagnosis of various cerebrovascular diseases (AVM, cancer, stroke). Recently, human studies using the new MT-balanced FENSI (Flow Enhanced Signal Intensity) technique[1,2] have shown reliable neuronal activation based on cerebral blood flux (CBFlux) in response to visual stimulation. Absolute CBFlux quantification is now possible with FENSI, leading the way to potential clinical applications. The objectives of this study are to assess the utility of the CBFlux biomarker by **1.**characterizing its dynamics in brain tumors and **2.**investigating the origins of the microvascular flux contrast obtained with FENSI. Specifically, we used FENSI to measure tumor blood flux (TBFlux) at different developmental stages of the 9L gliosarcoma in the rat brain. In addition, CBFlux and TBFlux measurements were compared with mean vessel density and fractional vascular surface derived from immuno-histochemistry, in different tumor regions and at different stages of tumor development.

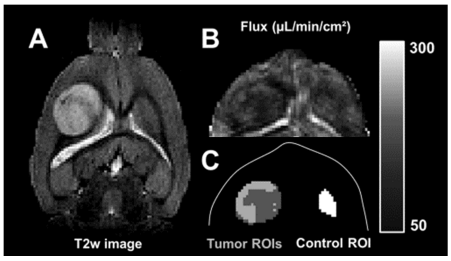

Figure 1 A. T2w image, **B.** parametric flux map and **C.** ROIs used for TBFlux/CBFlux measurements on rat #2 at Day 12.

Methods: All MR acquisitions were performed on a horizontal bore, 7T preclinical system. 15 300g male Fischer rats were used in agreement with the local ethics committee. One animal was used to verify that, in the current FENSI implementation[2], the MR signal was not contaminated by MT effects[3]. 14 rats were injected with 5 µL of 9L glial cells (~10^5) in the left striatum. For imaging, the animals were anesthetized using isoflurane [2% in air] and maintained still at 37°C. T_2 weighted images were acquired using a Fast Spin-Echo sequence (TR/TE=3000/56 ms, NX/NA=8/4, res. 200x200x500 µm, TA = 3min12s) to localize the tumor and the slice of interest (Fig. 1A). Absolute CBFlux/TBFlux quantification (Fig. 1B) was performed using a SE-EPI (TR/TE=6000/13 ms, res. 250x280x1000 µm, 75 pairs of control/tag, TA=60min) implementation of the MT-balanced FENSI technique[2], with imaging parameters (saturation duration and thickness) adapted to sensitize the acquisition to blood flow in the capillary network (0.5-2 mm/s). To characterize the temporal evolution of TBFlux, 10 rats were scanned both at early (Day 5-9) and late tumor developmental stage (Day 10-14). We performed histology on 11 animals (4//7 at Day 7//13-14). Two consecutive histological slices were treated with H&E and CD31&DAPI staining, respectively. For each rat, the density (µVD, mm^{-2}) and area (µVA, %) of CD-31 immuno-positive microvessels were automatically assessed in four ROIs randomly chosen on H&E staining. TBFlux, µVD and µVA ratios (rTBFlux, rµVD, rµVA) between areas of high and low TBFlux in the tumor (compartmentalized using minimum inter-class variance segmentation algorithm, ROIs in Fig. 1C) were tested for linear statistical dependence.

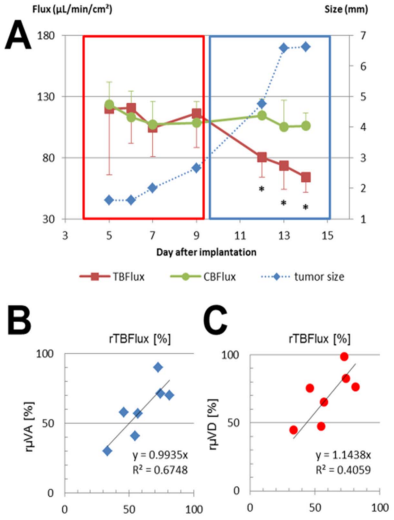

Figure 2 A. Temporal evolution of tumor size, TBFlux and CBFlux on 10 rats. Results show no statistical difference for small tumors (size < 3mm, red area) and significantly lower TBFlux for large tumors (tumor size > 3mm, blue area). TBFlux, µVA and µVD ratios in the tumor highlight significant statistical linear dependence between **B.**rTBFlux and rµVA (R^2=0.68) and **C.**rTBFlux and rµVD (R^2=0.41). Each rhombus and circle corresponds to a measurement performed on a single animal. On both graphs are also displayed the best linear approximations of the experimental data and their mathematical expressions.

Results: 1. Negligible MT effects were found in cortex and striatum, allowing for absolute flux quantification. Group analysis on early FENSI measurements (Day 5-9, n=14) showed no significant difference between CBFlux and TBFlux (p=0.3). When the tumor size exceeded 3 mm (Day 9-14, n=10), a significant microvascular flux decrease (38%, p<0.01) was found in the tumor region. The data showed reproducibility of FENSI measurements and heterogeneity of flux in tumor ROIs (Fig. 2A). **2.** Segmented FENSI flux maps delineated low-flux areas matching the initial 9L implantation site and higher vascularized regions around the tumor periphery. rTBFlux and rµVA were found strongly correlated at late developmental stage (R^2=0.68, Fig. 2B). rTBFlux and rµVD were also found correlated but to lesser extent (R^2=0.41, Fig.2C). The best linear approximations on both the rTBFlux/rµVA and rTBFlux/rµVD relationships presented a slope in a range close to unity (0.99 ± 0.17 and 1.15 ± 0.22). Preliminary results on large necrotic areas showed hypo-intense signal on FENSI parametric flux (n=2). Fluorescence microscopy on smaller tumors (Day 7, n=4) already revealed spatial heterogeneity of µVD and µVA inside the tumor. However, no relevant information could be obtained from the FENSI CBFlux maps inside the tumor, due to poor spatial resolution.

Discussion/Conclusion: 1. This study shows the feasibility of applying the FENSI technique to longitudinally and quantitatively characterize brain microvasculature *in vivo*. FENSI results are consistent with conventional 9L perfusion measurements performed with ASL, DSC-MRI or autoradiography, reporting respectively 53, 42 and 60% lower TBF in 9L gliosarcomas compared to normal CBF. Differences between TBF and TBFlux can however arise from flux orientation, media tortuosity, distorted pathways or partial volume effects. **2.** TBFlux-based tumor segmentation revealed typical 9L growth patterns. Linear positive correlation TBFlux/µVA/µVD suggests that the regions of high/low TBFlux in the tumor can directly reflect the neovascularization/necrotic processes associated with grade IV tumors, although direct comparison between FENSI and histochemistry is hindered by differences in spatial resolution, slice thickness and signal distortions. Using higher spatial resolution, absolute quantification of flux at microvascular level can provide a non-invasive insight into endothelial channels formation and microvessels orientation. Longitudinal FENSI studies can help to better understand and follow the mechanisms of neovascularization and cellular hypoxia associated with the angiogenic switch during tumor development. Moreover, FENSI can offer an alternative to ASL when studying ischemia or muscular diseases, because of its reduced sensitivity to transit times, labeling locations and disturbed pathways.

References: [1] Sutton BP, Ouyang C, Ching B, Ciobanu L. Functional imaging with FENSI: Flow-ENhanced Signal Intensity. Magnet Reson Med. 2007; 58. [2] Ouyang C, Sutton BP. Localized blood flow imaging using quantitative flow-enhanced signal intensity. Magnet Reson Med. 2012; 67: 660-668. [3] Reynaud O, Ciobanu L. Post-Processing Correction of Magnetization Transfer Effects in FENSI Perfusion MRI Data. Magnet Reson Med 2011; 65:457-462.